

### AMENDMENTS TO THE SPECIFICATION

**Please amend paragraph [0035] on page 13 as follows:**

[0035] It has been reported that ROS enhanced Mac-1 upregulation and anti-oxidants diminished Mac-1-mediated neutrophil accumulation and adhesion following ischemia and reperfusion; (Serrano et al., 1996; Fraticelli A, Serrano CVJ, Bochner BS, Capogrossi MC and Zweier JL, *Biochim Biophys Acta* **1310**:251-259,1996). In this study, ROS ( $\Theta_2^{\oplus-}$   $\underline{\Theta_2^{2+}}$  and  $H_2O_2$ ) production induced by fMLP was diminished by SPRST as well as Tet and Fan (Fig. 4). This indicates that SPRST, Tet, and Fan may act as ROS scavengers through which in turn down-regulate Mac-1 expression and then neutrophil firm adhesion/ transmigration. Our prior studies confirmed that antioxidants (superoxide dismutase and catalase) significantly down regulated ROS production as well as Mac-1 expression and neutrophil adhesion to fibrinogen (Shen YC, et al., *Eur J Pharmacol* **343**:79-86,1998). The flow cytometric method used in this study for the measurement of ROS production enabled on-line monitoring of the intracellular accumulation of  $\Theta_2^{\oplus-}$   $\underline{\Theta_2^{2+}}$  and  $H_2O_2$  in neutrophils. We found accumulation of  $\Theta_2^{\oplus-}$   $\underline{\Theta_2^{2+}}$  and  $H_2O_2$  began immediately after stimulation (data not shown). Thus, the rapid accumulation of  $\Theta_2^{\oplus-}$   $\underline{\Theta_2^{2+}}$  and  $H_2O_2$  in response to stimulation and our observation that Mac-1 upregulation could be inhibited by ROS scavengers (Shen et al., 1999) suggests that ROS are early signaling molecules involved in the regulation of neutrophil function. This argument is further intensified by Finkel's observations (Finkel T, *Curr Opin Cell Biol* **10**:248-253, 1998) that ROS can act as second messengers in the activation of ligand-stimulated NF- $\kappa$ B, various protein kinase C (PKC) family members, and mitogen-activated protein kinase (MAPK) as well as tyrosine kinases/phosphatase. Thus, we suggest that ROS could regulate neutrophil functions through second messenger mechanism(s).

**Please amend paragraph [0046] on page 18 as follows:**

[0046] SPRST, Tet, and Fan inhibited intracellular ROS ( $\Theta_2^{\oplus-} \underline{O_2^{2+}}$  and  $H_2O_2$ ) production. It has been shown that ROS (e.g.,  $\Theta_2^{\oplus-} \underline{O_2^{2+}}$  and  $H_2O_2$ ) upregulates Mac-1 expression and enhances neutrophil adhesion that could be abolished by antioxidants (Serrano et al., 1996; Fraticelli et al., 1996). Therefore, we hypothesized the de novo production of ROS by neutrophils may participate in Mac-1 upregulation that could be diminished by SPRST. We used a flow cytometric method to measure intracellular ROS production in fMLP-stimulated neutrophils in the presence or absence of SPRST. A representative experiment by fMLP-stimulated accumulation of intracellular  $H_2O_2$  (measured as DCF fluorescence) and  $\Theta_2^{\oplus-} \underline{O_2^{2+}}$  (measured as EB fluorescence), respectively, were illustrated in Fig. 8 & Fig. 9 while the results of five experiments are summarized in Fig.10). SPRST, Tet, and Fan concentration-dependently decreased the fluorescence intensity of EB and DCF induced by fMLP (Fig. 10,  $P < 0.05$ ,  $n=5-8$ ).

**Please amend paragraph [0050] on page 19 as follows:****[0050] EXAMPLE 1**

Radix Stephaniae tetrandrae (100 g) was milled and extracted with 95% EtOH three times at  $80 \pm \underline{^\circ C}$  (each 1000 ml, 8 h). The combined extract was concentrated by rotary evaporation in vacuum at  $50 \pm \underline{^\circ C}$  to dryness.

**Please amend paragraph [0051] on page 20 as follows:****[0051] EXAMPLE 2**

Radix Stephaniae tetrandrae (100 g) was milled and extracted with dichloromethane three times at  $80 \pm \underline{^\circ C}$  (each 1,000 ml, 8 h). Then extracted with MeOH at  $80 \pm \underline{^\circ C}$  (1,000 ml, 8h). The combined extract was concentrated by rotary evaporation in vacuum at  $50 \pm \underline{^\circ C}$  to dryness.

**Please amend paragraph [0052] on page 20 as follows:**

[0052] EXAMPLE 3

Radix Stephaniae tetrandrae (100 g) was milled and extracted with 95% EtOH two times at 80± °C (each 1,000 ml, 8 h). Then extracted with MeOH at 80± °C (1000 ml, 8 h). The combined extract was concentrated by rotary evaporation in vacuum at 50± °C to dryness.

**Please amend paragraph [0053] on page 20 as follows:**

[0053] EXAMPLE 4

Radix Stephaniae tetrandrae (100 g) was milled and extracted with 95% EtOH two times at 80± °C (each 1,000 ml, 8 h). Then extracted with dichloromethane at 80± °C (1,000 ml, 8 h). The combined extract was concentrated by rotary evaporation in vacuum at 50± °C to dryness.

**Please amend paragraph [0054] on page 20 as follows:**

[0054] EXAMPLE 5

Radix Stephaniae tetrandrae (610 g) was milled and extracted with 95% EtOH three times at 80± °C (each 1,000 ml, 8 h). The combined extract was concentrated by rotary evaporation in vacuum at 50± °C to dryness. 3% HCl (200ml) solution was added to the residue, then extracted with CHCl<sub>3</sub> (200 ml × 3).

RST extracted by water only; RST/H<sub>2</sub>O/EtOH, RST residue extracted by ethanol after water extraction; RST/EtOH, RST extracted by ethanol only; SPRST/EtOH/H<sub>2</sub>O, RST residue extracted by water after extraction with ethanol; RST/EtOH/CH<sub>2</sub>Cl<sub>2</sub>, RST residue extracted by CH<sub>2</sub>Cl<sub>2</sub> after extraction with ethanol; RST/CH<sub>2</sub>Cl<sub>2</sub>, RST extracted by CH<sub>2</sub>Cl<sub>2</sub> only; RST/CH<sub>2</sub>Cl<sub>2</sub>/EtOH, RST residue extracted by ethanol after CH<sub>2</sub>Cl<sub>2</sub> extraction; RST/CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>O, RST residue extracted by water after CH<sub>2</sub>Cl<sub>2</sub> extraction.

**Please amend the paragraph beginning at page 21, line 13 as follows:**

Radix Stephaniae tetrandrae (610 g) was milled and extracted with 95% EtOH three times at 80 °C (each 1000 ml, 8 h). The combined extract was concentrated by rotary evaporation in vacuum at 50 °C to dryness. 3% HCl (200ml) solution was added to the residue, then extracted with CHCl<sub>3</sub> (200 ml × 3). The acid solution was adjusted to pH 9 with 25% NH<sub>4</sub>OH and the resultant suspension were extracted with CHCl<sub>3</sub>. The CHCl<sub>3</sub> layer was evaporated to give tetrandrine and fangchinoline. The NH<sub>4</sub>OH layer was then partitioned with n-BuOH. The n-BuOH layer was concentrated and a residue (5.1 g) was chromatographed on Sephadex LH-20 column with MeOH to give three fractions (I, II, III). Fraction II, which contained mostly cyclanoline, was recrystallized with MeOH to give cyclanoline as gray-white power. The mother liquid of fraction II and fraction III were combined and were subjected to column chromatography over silica gel eluting with CHCl<sub>3</sub>-MeOH (9:1) to afford cyclanoline and oblongine. The four alkaloids were identified by comparing the IR, MS, <sup>1</sup>H- and <sup>13</sup>C- NMR spectral data with the literature data.<sup>4, 14-15.</sup>

**Please amend the paragraph beginning at page 21, line 32 as follows:**

A 100 g pulverized Radix Stephaniae tetrandrae was extracted five times with EtOH (1,500 ml, successively) by reflux at 80 °C each 5 h. The extracts were combined and filtered, then evaporated in vacuum at ca. 50 °C to give a 10.23g of residue. A 30mg of dried extract was dissolved in 1.5 ml of MeOH. The solution was filtered through a 0.45 μm syringe filter (Gelman Sciences, Ann Arbor, MI, USA) before use.

**Please amend paragraph [0062] on page 25 as follows:**

[0062] **Flow cytometric Analysis of Intracellular ROS Production.** Intracellular production of  $\text{O}_2^{\cdot -}$  and  $\text{H}_2\text{O}_2$  were measured as ROS production in this study and analyzed on a flow cytometer (FACSort; Becton Dickinson) according to our previous work (Shen et al. 1998).

Briefly, neutrophils ( $1 \times 10^6$  cells/ml) were incubated at  $37^\circ\text{C}$  for 5 min with  $20\ \mu\text{M}$  2',7'-dichlorofluorescein diacetate (DCFH-DA; Molecular Probes, Inc., Eugene, OR) and for an additional 15 min with  $10\ \mu\text{M}$  of hydroethidine (Molecular Probes). The acetate moieties of DCFH-DA are cleaved off intracellularly by esterases, liberating the membrane impermeable 2',7'-dichlorofluorescein, which fluoresces when oxidized to 2',7'-dichlorofluorescein (DCF) by  $\text{H}_2\text{O}_2$ ; hydroethidium, on the contrary, can be directly oxidized by  $\Theta_2^{\cdot-}$   $\text{O}_2^{2+}$  to ethidium bromide (EB), which fluoresces after intercalating with nucleic acids. After labeling, cells were pretreated with SPRST or other chemicals for 10 min and stimulated with fMLP ( $1\ \mu\text{M}$ ). Production of  $\Theta_2^{\cdot-}$   $\text{O}_2^{2+}$  and  $\text{H}_2\text{O}_2$  was then determined 30 min after on a flow cytometer (FACSort; Becton Dickinson) by measuring emission at 525 nm (FL1) for DCF and 590 nm (FL2) for EB. Data are expressed as mean channel fluorescence.